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EDITORIAL COMMENT

DNA Structural Variants as Genetic Risk Factors for the Long QT Syndrome*

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The long QT syndrome (LQTS) is a rare, inborn heart condition in which delayed repolarization of the heart following a heartbeat increases the risk of episodes of torsades de pointes (1,2). These episodes may be provoked by numerous stimuli and, in extreme cases, may lead to sudden death due to ventricular fibrillation (3,4).

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The study by Barc et al. (5) in this issue of the *Journal* illustrates the promise of using genetic information for disease prediction and prevention. Determining whether deoxyribonucleic acid (DNA) sequence polymorphisms or DNA structural variants are associated with subclinical markers (e.g., electrocardiogram [ECG] pattern) or clinical risk of syncope or cardiac arrest in otherwise healthy individuals would be of great importance. Barc et al. (5) provide evidence that DNA deletions ranging in size from ~10 to 650 kb can lead to both subclinical and clinical abnormalities; however, this direct link between DNA structural variants and clinical phenotype may be largely limited to rare (and relatively large) genetic rearrangements. Common structural variants will likely have more subtle effects and be more difficult to interpret.

LQTS has been shown to aggregate in families by careful clinical observation and longitudinal studies (6). In family studies, the LQTS inheritance pattern has been shown to be consistent with either an autosomal dominant (transmitted from parent to child, requiring 1 variant allele) or an autosomal recessive (appearance in families with carrier parents, requiring 2 variant alleles) locus. These mutations tend to prolong the duration of the ventricular action potential, lengthening the QT interval, and corresponding to the pattern seen on the ECG (1,2).

A number of genes have been identified in which mutations lead to LQTS, with no single mutation in a gene responsible for a significant number of cases. This indicates that there may be many rare, as-yet-unidentified functional variants within LQTS susceptibility genes. For example, LQT1 (the most common type of LQTS, between 30% and 35% of all cases) is caused by loss-of-function mutations in the *KCNQ1* gene on chromosome 11p15.5 that encodes the alpha subunit of the slow delayed rectifier potassium channel (7). These mutations often cause LQTS by reducing the amount of repolarizing current, leading to an increase in the action potential duration, and tend to be the most common yet least severe. LQT2 (the second most common form of LQTS, between 25% and 30% of all cases) is caused by mutations in the *KCNH2* (*HERG*) gene on chromosome 7q36.1 that encodes for the pore-forming alpha subunit of the rapid delayed rectifier potassium channel (8). The third most common gene involved in LQTS is *SCN5A* (LQT3), the alpha subunit of the voltage-gated type V sodium channel located on chromosome 3p21. Whereas LQT1, LQT2, and LQT3 account for over 90% of all mutations identified in LQTS cases, there are 9 additional LQTS genes that can cause altered ion-channel currents, and more than 600 different mutations have been identified among the 12 known genes (9). For many patients with LQTS, however, the underlying cause remains to be determined.

In their study, Barc et al. (5) hypothesized that the remaining, unknown genetic forms of LQTS could be, in part, determined by DNA structural variants (specifically copy number variants, or CNVs) rather than DNA sequence variants (such as single nucleotide polymorphisms, or SNPs). Earlier reports suggested that large deletions (10) or duplications (11,12) could lead to LQTS. In order to test this hypothesis, Barc et al. (5) investigated the role of rare deletions and duplications in LQT1 (*KCNQ1*) and LQT2 (*KCNH2*) in 93 cases with LQTS for which sequence variants in the exons and intron/exon boundaries for LQT1, LQT2, and LQT3 could not be identified by either denaturing high-performance liquid chromatography (dHPLC) or Sanger sequencing.

The value of genetics in identifying the molecular defects responsible for rare, single-gene (Mendelian) disorders is beyond dispute. In many examples (such as neonatal diabetes), the molecular diagnosis has led to both novel treatments and improved diagnosis/prediction. Even within the Mendelian diseases, there can be multiple sites of sequence variation that lead to the clinical outcome, with different sites often resulting in variation in disease severity. The number of single-gene disorders account for a small proportion of the overall burden of disease with a genetic component. In the case of LQTS, 3 distinct genes account for ~90% of the mutations identified in patients, thereby making each defined as a "single gene" disorder. Nonethe-

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less, there are "missing" genetic etiologies that lead to LQTS in a substantial proportion (~10%) of patients.

In the paper by Barc et al. (5), the diagnosis of LQTS was based upon the ECG (heart rate–corrected QT [QTc] duration and T-wave morphology) as well as clinical and family history, with duplicate ECG readings and full medical examination to exclude other forms of QT prolongation and misclassification in the genetic analysis. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed to screen for CNVs affecting *KCNQ1*, *KCNH2*, and *SCN5A*, and genome-wide array comparative genomic hybridization (CGH) was used to map CNVs identified by MLPA to higher resolution. In the 93 cases, 1 heterozygote deletion in LQT1 (*KCNQ1*) and 2 heterozygote deletions in LQT2 (*KCNH2*) were observed, with no structural variation identified in *SCN5A*.

Identification of deletions in 3 of 93 cases (probands) led to recruitment of family members for subsequent evaluation for evidence of transmission of the deletions and correlation with LQTS-related phenotype. One proband (heterozygote carrier, Family 1) had a 650-kb deletion that extended from exon 4 through the 3′ region in *KCNH2*. This CNV was present in 6 family members with ECG findings consistent with LQTS (based upon QTc interval) but who were asymptomatic at time of examination. The CNV was absent in 9 family members, all of whom had normal ECG reports. In this family, the presence of the CNV (deletion) is highly correlated with subclinical (ECG) phenotype, although clinical evidence remains to be determined.

A second proband (Family 2) also had a heterozygote deletion of *KCNH2*, this time of size 145 kb. Four family members (mother, brother, 2 sons) were examined for evidence of transmission of the deletion and phenotypic correlates of LQTS. Both the mother and the brother carried the deletion; however, the mother had prolongation of the QTc interval, whereas the brother had a normal ECG. The third proband (Family 3) had a small, imprecisely mapped deletion (2 to 14 kb) affecting exons 7 to 8 of *KCNQ1*. Three relatives (father, mother, and sister) were examined, with only the father having evidence of the deletion but also a borderline LTQS phenotype (suggestive T-wave pattern and QTc of 438 ms). Both the mother and sister failed to carry the deletion and had normal ECG readings. In these 2 families, the presence of the deletion is not highly predictive of either subclinical (ECG) or clinical phenotypes.

Recent studies have demonstrated that CNV in the human genome is extensive and could play an important role in susceptibility to disease, particularly in autism (13) and schizophrenia (14). A region on chromosome 16p11.2 was unique in that it had multiple de novo CNVs yet no evidence of inherited risk for autism, and a 593-kb deletion identified 2 candidate genes: *ATP10A* and *GABRB3*. Deletions and duplications larger than 100 kb also have been identified from 150 individuals with schizophrenia and 268 ancestry-matched controls (15). Novel deletions and duplications of genes were present in 5% of controls compared with 15% of all cases (and in 20% of young-onset cases, those thought to be more severely affected). Mutations in cases disrupted genes disproportionately from pathways controlling neurodevelopment, including neuregulin, ERK/MAPK signaling, synaptic long-term potentiation, axonal guidance, and glutamate receptor pathways. Thus, just as for sequence variation in candidate genes, analysis of structural variation can lead to biologically meaningful insights on disease mechanism.

Despite the considerable promise of structural variation being associated with disease, CNVs are not straightforward to genotype, and current methods to examine structural variation genome-wide (rather than in specific gene regions) are rapidly evolving (16,17). Structural variation (which includes duplications, deletions, and balanced rearrangements) has been shown to exist of all sizes and classes, and small CNVs have been under-represented due to the technical difficulties inherent in discovering and genotyping these variants. As noted by Barc et al. (5), the CNV sizes detected are variable (10 to 650 kb) with detectable effects on ECG (but not necessarily on clinical outcome). This result is consistent with the recent prediction that, for complex phenotypes, the contribution to disease susceptibility by CNVs may be small (17). This prediction is supported by the findings of the Wellcome Trust Case Control Consortium (18) who, in a study of 8 common diseases, identified just 3 common CNVs associated with disease, all of which had been previously identified by SNP-based mapping.

The significance of CNVs on risk of LQTS remains unclear. Barc et al. (5) suggest that CNV typing in *KCNQ1* and *KCNH2* be considered part of a "routine work up of LQTS in the absence of point mutations in the 3 major LQTS genes." This recommendation should be considered in the context of other (unknown) variants and the limitations of the current study. First, there is clear rationale for screening LQT1, LQT2, and LQT3 for DNA sequence variants contributing to LQTS, as these 3 genes account for ~90% of the patients identified with risk variants. The manner in which the screening occurs (now and in the future) provides a foundation for any recommendation. Rather than focusing purely on exons or intron/exon boundaries by dHPLC or Sanger sequencing, other approaches should be employed. Targeted sequencing has become common and is rapidly decreasing in cost, so capturing the entire gene and screening the complete sequence could identify both DNA and structural variants. This transition from inspection of "pieces" of genes to "complete" genes would provide near-complete genetic information. Of additional interest is the identification of all genetic contributors to LQTS. As noted in the previous text (17), one can not only survey the entire genome for CNVs, but also characterize those CNVs that are of much smaller size. This survey could assist in the detection of novel genes contributing to the residual LQTS genetic risk. As whole genome

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approaches become available, the cost and utility of DNA sequencing to detect both sequence and structural variation contributing to LQTS becomes feasible.

The practice of using genetic information to improve risk prediction is progressing at a rapid pace. In order to demonstrate the utility of genetic information for prediction, the genetic risk factors should be required to improve upon traditional risk factors. To date, the improvement of genetic risk for common, complex disease has been subtle. In the LQTS, the disease is relatively uncommon, and the genetic information correlates highly with risk and, in some cases, efficacy of treatment or intervention. In the Barc et al. (5) family collection, there is an opportunity to practice "personalized medicine" as members of 3 families have been identified as carriers of a CNV that (at least in 1 family) tracks with a LQTS ECG pattern. The transition from discovery of genetic variation to clinical practice, especially in asymptomatic individuals (based upon QT interval or T-wave morphology) is a topic with significant medical implications.

In summary, Barc et al. (5) have studied 93 cases of LQTS that lack DNA sequence variants that could be predictive of the disease by standard genotyping methods. In 3 cases, a heterozygote deletion (forming a copy number variant, CNV), in LQT1 and LQT2 was identified. In all 3 families, the CNV was transmitted to other family members; however, there were differences between the presence of CNV and the LQTS ECG pattern. This study raises new questions regarding the biological mechanisms of LQTS and the relationship between disrupted gene structure and function. Future studies examining the role of deleted or mutated genetic material on the function of ion channels are warranted. These studies may lead to novel insights on the manner in which the channels are regulated and provide the field with additional, predictive biomarkers that can be used with the ECG as predictors of cardiac arrest and sudden death.

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